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Regulatory role for hepatic low density lipoprotein receptors *in vivo* in the dog

(cholesterol synthesis inhibitor/mevinolin/bile acid sequestrant/colestipol/catabolism of plasma lipoproteins)

PETRI T. KOVANEN, DAVID W. BILHEIMER, JOSEPH L. GOLDSTEIN, JOHN J. JARAMILLO, AND MICHAEL S. BROWN

Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Contributed by Joseph L. Goldstein, October 17, 1980

ABSTRACT **Liver membranes from young beagle dogs were found to possess binding sites that resemble the low density lipoprotein (LDL) receptors originally described in cultured human fibroblasts. Treatment of the dogs with colestipol (a bile acid sequestrant) and mevinolin (a cholesterol synthesis inhibitor) produced a 3-fold increase in LDL binding activity. This increase correlated with a 2-fold increase in the fractional catabolic rate for intravenously administered human or canine ^{125}I-labeled LDL, suggesting that the increased hepatic receptors were responsible for the enhanced clearance of LDL from plasma. The hepatic lipoprotein receptors of control and drug-treated dogs resembled human fibroblast LDL receptors in that they bound apoprotein E-containing lipoproteins, such as very low density lipoproteins and a subfraction of high density lipoproteins (HDL_1), with 10-fold higher affinity than the apoprotein B-containing lipoprotein LDL; failed to bind canine HDL_2 and human HDL_3, which are devoid of apoproteins B and E; failed to bind methylated LDL; required calcium; and were destroyed by Pronase. Treatment of dogs with mevinolin not only increased the fractional catabolic rate for LDL but also reduced the synthetic rate for the lipoprotein. The current data suggest that the liver of dogs contains functional LDL receptors that are susceptible to metabolic regulation and that a drug-induced increase in the activity of these receptors can contribute to a lowering of plasma levels of LDL–cholesterol.**

The existence of the low density lipoprotein (LDL) receptor pathway for LDL degradation was first recognized in studies of an extrahepatic cell, the cultured human fibroblast (1). These cells possess a receptor that binds LDL at the cell surface and that facilitates its uptake by receptor-mediated endocytosis and its degradation in lysosomes, supplying cholesterol for cellular use. In addition to LDL, which contains apoprotein B, the LDL receptor binds lipoproteins that contain apoprotein E (2). The receptor has been described on various extrahepatic cells, both freshly isolated and maintained in culture, from man and animals (3). In each of these cell types, the number of receptors is regulated so that the cells increase their receptor production when cellular demands for cholesterol increase (1, 3). That LDL receptors contribute to the removal of LDL from plasma in man has been inferred from studies of patients with homozygous familial hypercholesterolemia. These subjects have a genetic deficiency of LDL receptors and, as a consequence, LDL accumulates in plasma, eventually producing atherosclerosis (1).

Inasmuch as the liver is the only organ that can excrete large amounts of cholesterol from the body, it is important to ascertain whether the liver produces LDL receptors and whether these receptors can serve as a route for the ultimate excretion of cholesterol in the form of either biliary cholesterol or bile acids. A receptor that resembles the functional LDL receptor of extrahepatic cells has been identified on the surface of parenchymal liver cells of intact rats (4–7). This receptor facilitates the hepatic uptake of LDL and very low density lipoproteins (VLDL) but not of typical high density lipoproteins (HDL) that lack apoproteins B and E.

The relevance of hepatic lipoprotein receptors to the control of plasma LDL levels in man has been underscored by the experiments of Shepherd *et al.* (8) with the drug cholestyramine. Cholestyramine is one of a class of nonabsorbable resins that bind bile acids in the intestine, carry them into the stool, and thus stimulate the hepatic conversion of cholesterol to bile acids (9, 10). This stimulation in turn leads to a lowering of plasma LDL–cholesterol levels. Shepherd *et al.* (8) showed that cholestyramine lowers LDL levels in man by increasing the rate at which LDL is removed from plasma through a receptor-mediated mechanism. This finding suggests that the liver may behave like cultured cells in that it develops an increased number of LDL receptors when the intracellular demand for cholesterol is enhanced (1), in this case by an increased demand for bile acid synthesis.

The current experiments were designed to answer three questions. Does the liver produce LDL receptors? Is the production of these receptors regulated by the liver's demand for cholesterol? Is an increase in hepatic LDL receptors associated with an enhanced rate of clearance of LDL from plasma? To study the regulation of the hepatic LDL receptor, we have used two drugs, colestipol and mevinolin, which, on the basis of theoretical considerations, should have a synergistic effect in enhancing the liver's demand for cholesterol. Colestipol is a bile acid-binding resin that produces an increased requirement for cholesterol for conversion to bile acids (9, 10). Mevinolin, like the related fungal metabolite compactin (11, 12), is a specific inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-controlling enzyme in cholesterol biosynthesis (13). Mevinolin was used to prevent the liver from developing an enhanced *de novo* cholesterol synthesis in response to colestipol, thus further increasing the liver's requirement for exogenous plasma cholesterol. The dog was chosen as a model for this study because dogs respond to both colestipol and mevinolin, either alone or in combination, with a reduction in plasma LDL levels (G. Kuron, J. Huff, and A. W. Alberts, personal communication; refs. 13 and 14).

MATERIALS AND METHODS

Materials. Mevinolin in the lactone form was provided by A. W. Alberts (Merck Sharp and Dohme Research Laboratories) (13). Colestipol was obtained from Upjohn.

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Abbreviations: FCR, fractional catabolic rate; HDL_1, a subfraction of canine high density lipoproteins that contains apoproteins A-I and E; HDL_2, the major fraction of canine high density lipoproteins that contains apoprotein A-I; LDL, low density lipoprotein; ^{125}I-LDL, ^{125}I-labeled LDL; VLDL, very low density lipoproteins.

Animals, Diets, and Drug Treatments. Male beagle dogs, approximately 3 months old and weighing 6–8 kg, were obtained from Marshall Research Animals (North Rose, NY). The animals were exposed to a light–dark cycle consisting of 12 hr of light (0600 to 1800) and 12 hr of darkness (1800 to 0600) for 1–2 weeks prior to use and during experiments. The dogs had continuous access to tap water and Wayne Lab Dog Diet (Allied Mills, Chicago). Mevinolin and colestipol were mixed with 50 g of Thoro-Fed dog food (Kal Kan Foods, Los Angeles) and administered orally every 12 hr (0700 and 1900) at the daily doses given in the legends. Control dogs were fed 50 g of Thoro-Fed without drugs.

Lipoproteins. Human LDL (ρ = 1.019–1.063 g/ml) was prepared from plasma by centrifugation (12). Human methyl-LDL was prepared by treatment of LDL with formaldehyde and sodium borohydride (15). Canine lipoproteins were isolated by a combination of centrifugation and Geon–Pevikon block electrophoresis as described (16). Each lipoprotein fraction was eluted from the Geon–Pevikon block with 150 mM NaCl and concentrated in a Pro-Di-Con apparatus (Bio-Molecular Dynamics, Beaverton, OR); each fraction migrated as a single band on agarose gel electrophoresis. The apoprotein content of each canine lipoprotein fraction, as judged by $NaDodSO_4$/polyacrylamide gel electrophoresis, was similar to that described by Mahley (2).

Lipoproteins were radioiodinated by the iodine monochloride method (17), dialyzed against 150 mM NaCl/0.01% EDTA, pH 7.4, and passed through a Millex-HA 0.45-μm filter (Millipore). For ^{125}I-labeled human LDL and canine LDL, an average of 2% and 10% of the radioactivity, respectively, was in lipid as determined by chloroform/methanol extraction. $NaDodSO_4$/polyacrylamide gel electrophoresis of canine ^{125}I-labeled LDL (^{125}I-LDL) (18) followed by scintillation spectrometry of the apoprotein bands showed that 97%, 2.5%, and 0.5% of the total ^{125}I radioactivity was contained in apoproteins B, A-I, and E, respectively. The average cholesterol/protein mass ratios for human LDL, canine LDL, canine HDL_1, and canine HDL_2 were 1.5, 0.8, 1.7, and 0.3, respectively. ^{125}I-Labeled canine apo-E-HDL_c (2) was provided by Robert Mahley. The concentration of each lipoprotein is expressed in terms of its protein content (19).

Preparation of Liver Membranes. Dogs were killed by an intravenous injection of 500 mg of sodium pentobarbital. Pieces of liver were immediately removed and placed in ice-cold 150 mM NaCl/1 mM phenylmethylsulfonyl fluoride. The liver was homogenized, and membranes (fraction sedimenting between 8,000 × *g* and 100,000 × *g*) were prepared in buffer A (150 mM NaCl/1 mM $CaCl_2$/1 mM phenylmethylsulfonyl fluoride/20 mM Tris·HCl, pH 8) as described (4, 6) except that the final pellet was subjected to an additional resuspension and resedimentation (100,000 × *g*, 60 min, 4°C). The pellets were assayed immediately or were rapidly frozen and stored in liquid nitrogen at −170°C. On the day of an experiment, the pellets were resuspended in buffer B (50 mM NaCl/1 mM $CaCl_2$/20 mM Tris·HCl, pH 8) by flushing five times through a 25-gauge needle. The suspensions were sonicated for 60 sec (4). The protein content of each suspension was determined (19), and the suspensions were diluted with buffer B to a final protein concentration of 5 mg/ml.

Binding of ^{125}I-Lipoproteins to Liver Membranes. The membrane binding of ^{125}I-lipoproteins was measured by an Airfuge centrifugation assay as described (4) except that the concentration of NaCl was reduced to 25 mM. The standard assay was conducted at a final pH of 8 in 80 μl of buffer C (25 mM NaCl/0.5 mM $CaCl_2$/50 mM Tris·HCl/20 mg of bovine serum albumin per ml) containing 100 μg of membrane protein and the indicated amount of canine ^{125}I-LDL (230–325 cpm/ng of protein) in the absence or presence of excess unlabeled lipoprotein. After incubation for 60–90 min at 0°C in an ice-water bath, membrane-bound ^{125}I-lipoprotein was separated from free ^{125}I-lipoprotein by centrifugation at 100,000 × *g* in a Beckman Airfuge with a 30° angle Airfuge rotor (4, 6). Samples were processed for scintillation spectrometry as described (4). All data points represent the average of duplicate assays.

Plasma Lipoprotein Turnover. Human ^{125}I-LDL (20 μCi; 330–490 cpm/ng of protein; 1 Ci = 3.7 × 10^{10} becquerels) was injected intravenously into a hind leg. Serial 2.5-ml blood samples were obtained during the ensuing 72 hr and were analyzed for ^{125}I radioactivity as reported (20). The plasma die-away curves were analyzed by a curve-fitting subroutine of the SAAM-25 program (21), and the kinetic parameters were calculated by the two-pool Matthews model (22). In control experiments we determined that the turnover of human ^{125}I-LDL, human ^{131}I-LDL, and canine ^{125}I-LDL in dogs was similar under the conditions of these experiments (see *Discussion*).

RESULTS

Membranes prepared from the livers of normal young beagle dogs bound canine ^{125}I-LDL with high affinity (Fig. 1). High-affinity binding was eliminated by an excess of unlabeled LDL or by 30 mM EDTA. Binding of canine ^{125}I-LDL to the liver membranes was inhibited competitively by human LDL as well as by canine LDL (Fig. 2*A*), but the human LDL was several-fold less effective. Reductive methylation of human LDL destroyed its ability to compete with canine ^{125}I-LDL (Fig. 2*A*). Binding of canine ^{125}I-LDL was also inhibited competitively by canine VLDL, which contains apoproteins B and E (Fig. 2*B*). Canine HDL_1, a subfraction of HDL that contains apoproteins E and A-I (2), also competed for the binding of ^{125}I-LDL. On the other hand, canine HDL_2, which contains apoprotein A-I but no apoprotein B or E, failed to compete with canine ^{125}I-LDL (Fig. 2*B*). Treatment of the liver membranes with 10 μg of Pronase per ml (25 min at 37°C) abolished high-affinity binding of canine ^{125}I-LDL (data not shown).

To study the regulation of the hepatic lipoprotein-binding site, we treated groups of dogs with oral colestipol and mevinolin either alone or in combination. After a steady-state level of plasma LDL was reached, dogs were injected intravenously with human ^{125}I-LDL, and the fractional catabolic rate (FCR) of the lipoprotein was calculated by the two-pool Matthews model. Finally, the animals were killed and liver membranes were prepared for measurement of ^{125}I-LDL binding activity. For comparative purposes, binding assays were performed at a

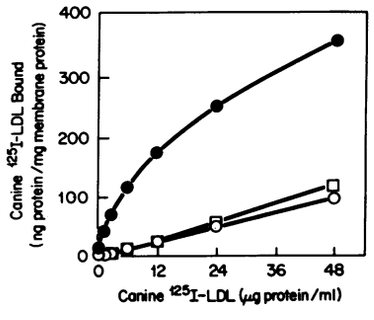

FIG. 1. Saturation curve for the binding of canine ^{125}I-LDL to liver membranes from a normal dog. Each assay tube contained the indicated concentration of canine ^{125}I-LDL in the absence (●) or presence of either 1 mg of protein per ml of unlabeled canine LDL (○) or 30 mM EDTA (□). The tubes were incubated for 90 min at 0°C.

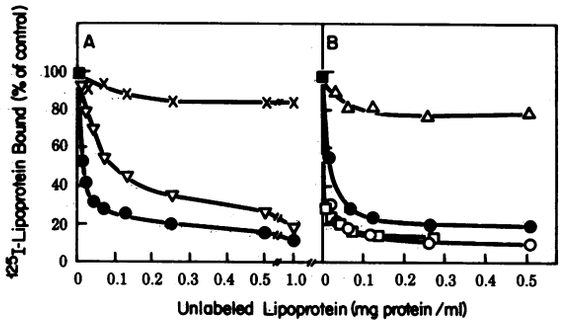

FIG. 2. Ability of unlabeled canine and human lipoproteins to compete with canine ^{125}I-LDL for binding to liver membranes from a normal dog. Each assay tube contained 3 μg of canine ^{125}I-LDL per ml and the indicated concentration of one of the following unlabeled lipoproteins: ▽, human LDL; ×, human methyl-LDL; □, canine VLDL; ●, canine LDL; ○, canine HDL_1; or △, canine HDL_2. The tubes were incubated at 0°C for 60 min. The "100% control" values for canine ^{125}I-LDL bound in the absence of unlabeled lipoproteins (■) were 40 and 45 ng/mg of protein in Exps. A and B, respectively.

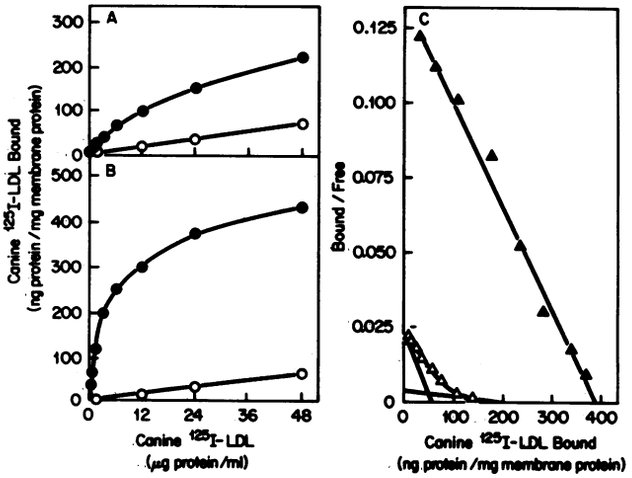

FIG. 3. Saturation curves for binding of canine ^{125}I-LDL to pooled liver membranes from three control dogs (*A*) and two dogs treated with mevinolin and colestipol (*B*). Treated dogs were given oral mevinolin (10 mg/kg per day) and colestipol (700 mg/kg per day) for 17 days. Each assay tube contained the indicated concentration of canine ^{125}I-LDL in the absence (●) or presence (○) of 1 mg of unlabeled canine LDL per ml. Tubes were incubated for 90 min at 0°C. (*C*) Scatchard plots (24) of the specific binding data, obtained by subtracting the amount of ^{125}I-LDL bound in the presence of excess unlabeled LDL from that bound in its absence. △, Control dogs; ▲, treated dogs. The curvilinear Scatchard plot of the control dogs (△) was resolved into two components by vectorial analysis (25).

canine ^{125}I-LDL concentration of 3 μg/ml, which is approximately equal to the K_d value for the receptor site at 0°C.

Colestipol at a dose of 700 mg/kg per day for 17 days reduced the plasma LDL level by about 25% to 16 mg/dl (Table 1). The FCR for the infused human ^{125}I-LDL rose by 28% from 1.63 per day in control dogs to 2.08 per day in treated dogs. Mevinolin at a dose of 10 mg/kg per day for 17 days reduced the plasma LDL level from 21 to 9 mg/dl and increased the FCR slightly to 1.95 per day. Low doses of mevinolin (10 mg/kg per day) and colestipol (700 mg/kg per day) reduced the LDL level to 6 mg/dl and also produced the highest FCR for human ^{125}I-LDL, the latter value increasing 2-fold over the control to 3.39 per day.

Specific binding of canine ^{125}I-LDL to liver membranes increased slightly from 34 to 46 ng/mg in dogs treated with colestipol. Mevinolin at 10 mg/kg per day increased the binding to 60 ng/mg (Table 1). The combination of mevinolin and colestipol further increased the specific binding of canine ^{125}I-LDL to 111 ng/mg, a value that was 3-fold higher than that of the control dogs. Similar increases in LDL binding were observed when the same canine liver membranes were assayed with human ^{125}I-LDL at 12.5 μg/ml. The correlation coefficient for canine compared to human ^{125}I-LDL binding in the various groups was 0.86 (data not shown). In control experiments, we found that membranes from unfractionated liver homogenates showed the same increase in ^{125}I-LDL binding as did the 8,000–100,000 × *g* pellet of membranes in the animals shown in Table 1.

In an additional experiment we administered a 2.5-fold higher dose of mevinolin (25 mg/kg per day) to three dogs for 23 days to determine whether such treatment would reproduce the response seen with the low dosage of mevinolin and colestipol (Table 1). In dogs treated with a high dose of mevinolin, plasma LDL declined to 6 mg/dl, which was the same as achieved with low doses of mevinolin and colestipol. However, hepatic binding of ^{125}I-LDL increased to only 68 ng/mg, and the FCR for ^{125}I-LDL increased to only 2.64 per day. Both of these values were lower than the ones produced by addition of colestipol to the low dose of mevinolin.

Table 1. Plasma lipoprotein cholesterol levels, ^{125}I-lipoprotein binding to liver membranes, and plasma turnover of ^{125}I-LDL in dogs treated with mevinolin and colestipol

Treatment				Plasma cholesterol, mg/dl				Specific binding of canine ^{125}I-LDL to liver membranes, ng/mg	FCR for human ^{125}I-LDL, day^{-1}	Relative LDL synthetic rate*
Drug	Dose, $mg \cdot kg^{-1} \cdot day^{-1}$	Duration, days	No. of dogs	Whole plasma	VLDL	LDL	HDL			
None	0	—	14	135 ± 9	1 ± 0.3	21 ± 2	104 ± 6	34 ± 3	1.63 ± 0.07	1.0
Colestipol	700	17	5	124 ± 8	3 ± 1.0	16 ± 1†	100 ± 6	46 ± 3	2.08 ± 0.16	1.0
Mevinolin	10	17	5	95 ± 9†	1 ± 0.2	9 ± 2†	77 ± 7†	60 ± 4†	1.95 ± 0.06	0.5
Mevinolin +colestipol	10 700	17	6	79 ± 6†	1 ± 0.3	6 ± 1†	68 ± 5†	111 ± 10†	3.39 ± 0.36†	0.6
Mevinolin	25	23	3	73 ± 11†	1 ± 0.3	6 ± 1†	62 ± 10†	68 ± 18†	2.64 ± 0.49†	0.5

Three days before the end of the treatment period, each dog received an intravenous injection of 20 μCi of human ^{125}I-LDL, and the FCR was measured. At the end of the treatment period, each dog was killed, the plasma lipoprotein–cholesterol content was measured by a combination of centrifugation and heparin/manganese precipitation (20), and liver membranes were prepared. For binding reactions, each assay tube contained 3 μg of canine ^{125}I-LDL per ml in the absence or presence of 1 mg of unlabeled canine LDL per ml. Tubes were incubated at 0°C for 60 min. Specific binding was calculated as described in the legend to Fig. 3. All values represent the means ± SEM of data obtained for the indicated number of dogs.

* The method for calculation of LDL synthetic rate is described in the *Discussion*.

† $P < 0.05$. Statistical analyses were performed by Kruskal–Wallis analysis of variance and the Newman–Keuls multiple comparisons procedure (23).

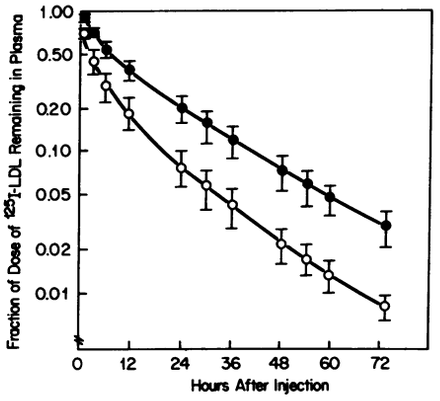

FIG. 4. Disappearance of human ^{125}I-LDL from the plasma of 14 control dogs (●) and 6 dogs treated with mevinolin and colestipol (○). Treated dogs received oral mevinolin (10 mg/kg per day) and colestipol (700 mg/kg per day) for 17 days. Three days before the end of the treatment period, each dog received 20 μCi of human ^{125}I-LDL intravenously. The fraction of the injected dose of ^{125}I-LDL remaining in the plasma is plotted semilogarithmically against time. Each value represents the mean ± SD of data obtained from 14 control dogs and 6 treated dogs.

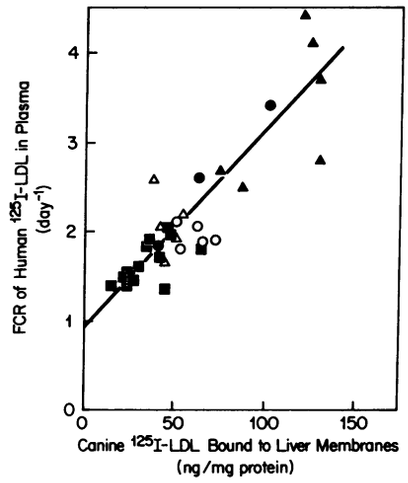

FIG. 5. Relationship between canine ^{125}I-LDL binding to canine liver membranes and the FCR for human ^{125}I-LDL in the plasma of intact dogs subjected to various treatments. ■, None (control); ○, mevinolin (10 mg/kg per day) for 17 days; ●, mevinolin (25 mg/kg per day) for 23 days; △, colestipol (700 mg/kg per day) for 17 days; ▲, mevinolin (10 mg/kg per day) plus colestipol (700 mg/kg per day) for 17 days. Measurements were made as described in the legend to Table 1, and a line was fitted to the points by the method of least squares. Each data point for specific membrane binding and plasma FCR was obtained on the same dog. $y = 0.022x + 0.93$; $r = 0.87$.

Fig. 3 *A* and *B* shows saturation curves for the binding of canine ^{125}I-LDL to pooled liver membranes from control dogs and dogs treated with mevinolin and colestipol. The combined drugs caused an increase in specific binding without affecting the nonspecific binding; i.e., the amount of ^{125}I-LDL bound in the presence of an excess of unlabeled LDL. A Scatchard plot of the specific binding data in the control animals (Fig. 3*C*) was curvilinear, which is compatible with the presence of high- and low-affinity binding sites. The K_d for the high-affinity site was 3.0 μg/ml, and the maximal binding was 55 ng of ^{125}I-LDL–protein bound per mg of membrane protein. In the dogs treated with mevinolin and colestipol, the K_d for the high-affinity site was unchanged at 3.6 μg/ml, but the maximal binding was increased to 390 ng/mg. In other experiments with different preparations of canine ^{125}I-LDL, the K_d for the high-affinity binding site ranged between 3 and 10 μg/ml.

Fig. 4 shows the mean disappearance curve for human ^{125}I-LDL from the plasma of 14 control dogs and 6 dogs treated with mevinolin and colestipol. In the treated dogs, the slopes of the first and second exponentials were both increased, and the calculated FCR was increased by 2-fold as compared with the control dogs.

A plot of the FCR for human ^{125}I-LDL against the amount of canine ^{125}I-LDL bound to liver membranes in the variously treated animals is shown in Fig. 5. A line could be fitted to these data by the method of least squares with a correlation coefficient of 0.87, suggesting a relationship between the increased FCR and the increased ^{125}I-LDL binding activity in liver membranes.

DISCUSSION

The current experiments were designed to evaluate the hypothesis that colestipol (a bile acid sequestrant) and mevinolin (a cholesterol synthesis inhibitor) would act synergistically in raising the level of LDL receptors in the livers of dogs. The results demonstrate that membranes prepared from livers of normal dogs contain high-affinity binding sites that resemble the LDL receptors previously characterized in cultured human and animal cells (1–3) and in membranes prepared from rat liver (4, 6) and adrenal glands from the cow, rat, and mouse (26, 27); the number of hepatic lipoprotein-binding sites increases in a synergistic fashion when dogs are treated with the combination of mevinolin and colestipol; and the increase in the number of canine hepatic binding sites measured *in vitro* after drug treatment correlates with an increase in the FCR for intravenously injected human LDL *in vivo*.

Like the LDL receptor of human fibroblasts (1, 2) and the lipoprotein receptor of rat liver (4, 6), the canine hepatic lipoprotein-binding site recognized lipoproteins containing apoprotein E with higher affinity than those containing only apoprotein B, and it did not bind lipoproteins that were devoid of apoproteins B and E. At 0°C the apparent K_d for canine LDL was 3–10 μg/ml (by both direct binding and competition studies), whereas the apparent K_d for canine VLDL was ≈0.3 μg/ml (by competition studies) and the apparent K_d for canine HDL_1 was also ≈0.3 μg/ml (by direct binding and competition studies). In experiments not shown, we found that canine ^{125}I-labeled apo-E-HDL_c, a lipoprotein that contains only apoprotein E (2), also bound with high affinity to the hepatic lipoprotein-binding site (K_d = 0.3 μg/ml). On the other hand, canine ^{125}I-HDL_2, which lacks apoprotein B and E, did not bind to this site, as determined by direct binding and competition studies. If this receptor functions *in vivo* like the LDL receptor of cultured cells, then it would be expected to promote the hepatic uptake of VLDL, VLDL remnants, HDL_1, apo-E-HDL_c, and LDL.

The synergism between colestipol and mevinolin in raising the hepatic lipoprotein receptors and increasing the FCR for LDL is illustrated by the data of Table 1. Mevinolin (10 mg/kg per day) increased hepatic ^{125}I-LDL binding by 26 ng/mg (the difference between 34 ng/mg in control animals and 60 ng/mg in treated animals). Colestipol alone increased ^{125}I-LDL binding by 12 ng/mg. When the two drugs were administered together, however, hepatic ^{125}I-LDL binding rose by 77 ng/mg, a value that is 2-fold higher than the value of 38 ng/mg, which would be expected if the actions of the drugs were additive. The synergism in elevating LDL receptors was mirrored by nearly identical synergism in elevating the FCR for LDL. Colestipol plus mevinolin increased the FCR by an amount that was 2.3-

fold higher than the predicted increase if the two drugs were additive in their effects.

Although the purpose of the current study was to investigate the receptor-mediated degradation of LDL, the data permit calculation of the relative synthetic rates for LDL in the variously treated animals. These calculations are based on the assumption that the measured changes in FCR for human ^{125}I-LDL correlate with similar changes in the FCR of the dog's own LDL. This assumption was validated in a series of studies in which we compared the decay rate of electrophoretically purified canine ^{125}I-LDL and human ^{131}I-LDL administered simultaneously to the same dogs. The calculated FCR for both lipoproteins agreed within 17% in two control dogs, three colestipol-treated dogs, and two dogs treated with colestipol and mevinolin.* By multiplying the FCR (day^{-1}) by the steady-state plasma LDL level ($mg \cdot dl^{-1}$) one can calculate a relative synthetic rate for canine LDL in units of $mg \cdot dl^{-1} \cdot day^{-1}$. These values are displayed in the extreme right column of Table 1, with the value in the control dogs (34 $mg \cdot dl^{-1} \cdot day^{-1}$) set equal to 1.0.

These calculations indicate that the low dose of mevinolin reduced the apparent synthetic rate for LDL by 50%, a reduction that was largely responsible for the 67% decline in LDL levels. When the dose of mevinolin was raised by 2.5-fold, the synthetic rate for LDL did not decrease further, but the LDL level dropped by an additional 33% owing to a further 35% increase in the FCR. Colestipol at the dose used did not affect the LDL synthetic rate but decreased the plasma LDL level slightly owing to a slight increase in the FCR. In the dogs that received low doses of mevinolin and colestipol, the plasma LDL level fell an additional 33% as compared with mevinolin alone, and all of this additional fall was attributable to an increase in the FCR. Considered together, these data suggest that mevinolin can lower the LDL synthetic rate by about 50% and that lowering the plasma LDL level below 50% of control values requires an increase in the FCR.

The increased FCR for ^{125}I-LDL achieved by mevinolin and colestipol was correlated with a proportionate increase in the number of high-affinity ^{125}I-LDL-binding sites in liver membranes (Fig. 5), suggesting that the enhanced clearance was due, at least in part, to an enhanced receptor-mediated uptake of LDL by the liver. Whether other tissues also developed an increased LDL receptor activity in these animals is not known.

Although the mechanism for the increased hepatic LDL receptor activity cannot be established unequivocally from studies of this type, the data are nevertheless consistent with the following model. Colestipol, by sequestering bile acids in the intestine, causes the liver to increase its cholesterol synthesis and also to develop a slight increase in LDL receptors. Mevinolin, by inhibiting the increased cholesterol synthesis, causes the liver to develop an even more pronounced increase in LDL receptor activity. The resulting combination of decreased LDL synthesis and more efficient LDL clearance leads to a profound 70% drop in plasma LDL levels.

As discussed above, the FCR for human and canine LDL were similar in the current studies even though the canine LDL had a 3- to 6-fold higher affinity for the hepatic LDL-binding site as compared with human LDL. An explanation for this apparent paradox was provided by an experiment in which the unidirectional rates of association and dissociation of the two lipoproteins from the hepatic binding site were measured at 37°C. At equal concentrations, the two lipoproteins associated with the receptor at similar rates; however, when association was terminated by addition of an excess of unlabeled LDL from the respective species, the human ^{125}I-LDL dissociated from the receptor at a rate that was 6-fold faster than the canine LDL. The time for 50% dissociation was 5 min and 28 min for human and canine ^{125}I-LDL, respectively. Inasmuch as receptor-bound LDL is internalized by cells within 5 min and before dissociation can occur (3), the association constant for various lipoproteins may be more important in dictating the relative rates of *in vivo* uptake than is the equilibrium binding constant.

Although it would be premature to generalize on the basis of these findings in young beagle dogs, the current studies raise the possibility that mevinolin and colestipol may have useful synergistic actions in lowering the plasma LDL level in man.

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* The ratios of the FCR for simultaneously injected human ^{131}I-LDL to the FCR for canine ^{125}I-LDL were as follows: 1.53/1.74 and 1.44/1.33 for two control dogs; 1.96/2.36, 2.18/2.27, and 1.61/1.77 for three colestipol-treated dogs; and 4.64/4.23 and 2.48/2.82 for two dogs treated with mevinolin and colestipol.